

***Trypanosoma (Megatrypanum) ornata* sp. n., a Parasite of the Eurasian Water Shrew
Neomys fodiens (Pennant, 1771)**

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Summary. Infection with the new trypanosome species *Trypanosoma ornata* sp. n. was detected in water shrews *Neomys fodiens* examined for presence of blood parasites in September 2003 and 2004 in Białowieża Forest (eastern Poland). The prevalence of infection was about 36.3%. The trypanosomes occurred as trypomastigote forms only, the epimastigote, dividing forms or other developmental stages were not observed. The morphological features of the species (big body size, small kinetoplast located close to the cell margin, the presence of vermiform posterior end, the surface of the cell markedly striated with longitudinal ‘myonemes’, short free flagellum) support its affiliation to *Megatrypanum* subgenus. The detail morphometric characterization is given.

Key words: *Neomys fodiens*; *Trypanosoma ornata*; Białowieża Forest

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INTRODUCTION

The trypanosomes parasitizing wild mammals in palearctic zone are not very well known. These are the species belonging to the Stercorarian group, from the subgenus *Megatrypanum*, *Herpetosoma* and *Schizotrypanum*. From salivarian trypanosomes few species, characterized with the cosmopolitan range are recorded. So far, 25 species of trypanosomes are noted as permanent components of parasitofauna of European mammals (Hoare 1972, Karbowiak et al. 2001). However, trypanosomes parasitizing insectivores were not investigated in Poland, also the records from other countries are poor and randomly examined. There is only a single report on the presence of a non-identified *Trypanosoma* in *Neomys fodiens*, which origins from England from the beginning of 20th century (Henry 1913).

MATERIALS AND METHODS

Eurasian water shrew *Neomys fodiens* (Insectivora: Soricidae) is a small (ca. 15 g of body mass), semiaquatic mammal occurring across most of Palearctics. It lives in wet habitats along fresh-waters and aquatic prey usually constitutes over 50% of its diet. During its short life (mean life-span 13.5 months), water shrew produces 2 or 3 litters of 6 or more (up to 15) young (Pucek 1981, Innes 1994, Rychlik, 2000).

The material from water shrews was collected in September 2003 and 2004 in Białowieża Forest (eastern Poland), in Knihiniówka area (ash-alder forest and sedge swamp, compartment 426). The shrews were caught in live-traps placed at permanent trap stations arranged in a rectangular grid or line. Traps were placed and set in the afternoon, checked four times per day, and blocked after a final check around midnight.

Blood samples taken from the tip of the tail were examined for the presence of trypanosomes using microhaematocrit centrifugation technique (8 minutes, 6200 g). They accumulated above the WBCs fraction, and their movements were observed under a light microscopy, using magnifications of 10 × 10 and 10 × 20 (eyepiece × objective). Giemsa-stained blood films were used for morphological and mensural characteristics of trypanosomes. Smears were made from the fraction containing trypanosomes. The volume of blood used was about 2 µl. Additionally, thin smears were prepared from fresh, non-centrifugated blood. All smears were air-dried, fixed in methyl alcohol and stained for 1 hour with Giemsa, diluted (1:5) in phosphate buffer, pH 7.2. Slides were rinsed, dried and

examined under a light microscopy using magnifications of 10×100 and 12×100 (eyepiece \times objective).

For the measurements of parasites, the “Analysis” software, in combination with a video camera and Olympus BX50F4 microscopy was used. This method provided possibilities for obtaining exact results accurate to $0.01 \mu\text{m}$. In order to detail, characterize, and compare morphological features of parasites, the terminology commonly adopted by trypanosome researchers (Hoare 1972, Matthews et al. 1977, Kingston et al. 1992) was employed in the present study. Morphometric measurements were made on 100 parasite specimens, at a magnification $1200\times$. Typical trypomastigote forms were selected for measurements. Weakly stained, damaged, or distorted individuals were ignored.

RESULTS

Infection with *Trypanosoma ornata* sp. n. was discovered in 4 out of 11 water shrews examined. Estimated prevalence of infection was about 36.3 %. Additionally, unidentified *Bartonella* sp. bacterias were detected in 5 shrews (prevalence about 45.4%), but there were no mixed infections. The trypanosomes in the blood of the water shrew occurred as trypomastigote forms only. The pleomorphism of the parasites was noticeable. The epimastigote, dividing forms or other developmental stages were not observed. The intensity of infection was low; only single parasites were visible in the capillars, the estimated number of parasites per 1 ml of blood, calculated on the base of blood smears was about 100-150, in single case about 500.

The trypanosomes occurred in thick-set, broad forms, in many cases with somewhat vermiform posterior end. A few specimens were characterized with less sharp or blunt posterior end. The body was slightly curved, with undulating membrane located on the external side of the curvature. The surface of the cell was markedly striated with dark-blue, longitudinal ‘myonemes’ (Fig. 1.). The length of the body was $20.50\text{--}40.90 \mu\text{m}$ (mean $29.43 \mu\text{m}$), the width was $1.93\text{--}4.12 \mu\text{m}$ (mean $3.06 \mu\text{m}$). The free flagellum was relatively short or absent, if present it varies in length from a mere stub to a short whip (mean $3.12 \pm 1.26 \mu\text{m}$). Some specimens had a longer flagellum, up to $9.18 \mu\text{m}$. The undulating membrane was weakly developed, adhering closely to the body, however it was always visible; its width was about $0.55\text{--}1.09 \mu\text{m}$. The nucleus was oval in shape and located in the middle or in the posterior part of the body (mean $\text{NI} = 0.69$, range $0.31\text{--}1.20$), along to the longitudinal axis of body, on the side of undulating membrane. It was $1.18\text{--}6.88$ (mean 3.78) μm long, and 0.65--

3.01 (mean 1.15) μm in width, on the Giemsa-stained smears it was violet coloured. The kinetoplast was particularly small. It was visible on the blood smears as oval in shape, $1.20 \times 0.60 \mu\text{m}$ in diameter, it usually is lying close to the margin of the undulating membrane and nucleus. It was located near the posterior end of the body or in the middle of PN distance (KI = 1.31–2.53, mean 1.68) (Fig. 1, Fig. 2). A wide range of morphometrics parameters (Table 1) was readily noted.

DISCUSSION

In the light of present knowledge, it is possible to find the representatives of two *Trypanosoma* subgenus – *Megatrypanum* and *Herpetosoma* in small mammals of Europe. According to Hoare (1972) the characteristic features of *Megatrypanum* trypanosomes are: relatively large size; kinetoplast situated close to the nucleus and far from the posterior end of body (index KI ≥ 2.0); reproduction in epimastigote stage in mammalian host; relatively wide body; short or absent free flagellum; small kinetoplast; organelles arranged along the longitudinal axis of the cell. The diagnostic features of trypomastigotes of *Herpetosoma* are: medium size; slender and curved body; long and pronounced free flagellum; large and often rod-shaped kinetoplast, lying nearer to the posterior extremity than to the nucleus (KI = 1.2–2.0, average 1.5); anterior nuclear position or in the middle of the body (Hoare 1972).

There are two species of *Trypanosoma* described in insectivores in Europe: *Trypanosoma talpae* Nabarro, 1907 and *Trypanosoma crocidurae* Brumpt, 1923. *T. talpae* is described from the mole *Talpa europaea* in western Europe and is included presently in the *Megatrypanum* subgenus (Hoare 1972, Baker 1974, Podlipaiev and Krylov 1990). Trypanosomes found in *N. fodiens* differed from *T. talpae* in some features: different localization of nucleus and kinetoplast in the cell, proportionally shorter PK and longer KN distances and a narrower body. *Trypanosoma crocidurae* is described from the blood of *Crocidura russula* and *Sorex araneus* in Belgium, Germany and France (Hoare 1972) as well as from *Crocidura suaveolens* in Czech (Šebek 1975). This species is included in the *Herpetosoma* subgenus; however, the classification based on the morphological analysis of data given in their descriptions by several authors and collected by Hoare (1972) can be discussed. The size of *T. crocidurae* (25–32 μm) is similar to the representatives of the *Herpetosoma* species, but the long PK distance (11 μm) and presence of the filiform “tail” at the posterior end relate this species to *Megatrypanum* subgenus. On the other hand, the only feature resembling this parasite to trypanosomes found in the present study in *N. fodiens* is

filiform “tail” on the posterior end of the body; the other parameters of these two species are different.

It is impossible to compare our results with the record of Henry (1913) on the presence of trypanosomes in *N. fodiens* in England due to the absence of any description.

The trypanosomes found in *N. fodiens* fulfil some of the conditions of subgenus *Megatrypanum*. The body size, and KI index smaller than 2.0 in the majority of specimens, support its affiliation to *Herpetosoma* subgenus, the most common in small mammals of Europe (Baker 1974, Hoare 1972, Karbowiak et al., 2001). However, in 10 % of specimens the value of KI index is bigger than 2, and the highest value of range is 2.53 (Table 1). Moreover, the other morphological features, as broad body, small kinetoplast, short free flagellum, presence of the vermiform “tail” and “myonemes”, the lack of free flagellum in some specimens, are characteristic of *Megatrypanum*.

In conclusion, the morphological and biological characteristics of *Trypanosoma ornata* sp.n. found in *N. fodiens* from Białowieża Forest place this parasite within *Megatrypanum* subgenus. Presented results are enough to give detail morphological description. However, only a few specimens of water shrews have been investigated, and developmental cycle of the parasite, its invertebrate vector and ecology remain unknown. Therefore, further investigations are needed.

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Table 1. Dimensions (in μm) of *Trypanosoma ornata* sp. n. from water shrew *Neomys fodiens* from Białowieża. Abbreviations: PK - posterior end to kinetoplast, KN - kinetoplast to nucleus centre, PN - posterior end to nucleus centre, NA - nucleus centre to anterior end, BL - body length, FF - free flagellum length, L - total length, NI - nucleus length, Nw – nucleus width, W - width of body on the nucleus level excluding the undulating membrane. Indices: nuclear index $\text{NI} = \text{PN} / \text{NA}$, kinetoplastic index $\text{KI} = \text{PN} / \text{KN}$, flagellar index $\text{FF}:\text{BL}$.

| Parameter | | PK | KN | PN | NA | BL | FF* |
|-----------|---------------|--------------|-------------|--------------|---------------|---------------|-------------|
| n=100 | mean | 4.27 ± 1.54 | 6.48± 1.43 | 10.75 ± 2.30 | 15.90 ± 2.32 | 26.66 ± 3.52 | 3.12 ± 1.26 |
| | range | 2.62 - 10.65 | 2.48 -14.35 | 5.41 - 18.84 | 10.97 – 21.01 | 20.50 - 38.26 | 1.00 - 9.18 |
| L | | NI | Nw | W | NI | KI | FF:BL* |
| mean | 29.43 ± 4.10 | 3.78 ± 0.85 | 1.15 ± 0.35 | 3.06 ± 0.43 | 0.69 ± 0.16 | 1.68 ± 0.24 | 0.10 ± 0.06 |
| range | 20.50 - 40.90 | 1.18 – 6.88 | 0.65 - 3.01 | 1.93 - 4.12 | 0.31 - 1.20 | 1.31 – 2.53 | 0.03 - 0.30 |

* - if the free flagellum is present

Figure legends:

Fig. 1. Photomicrograph of bloodstream forms of *Trypanosoma ornata* sp. n. from water shrew *Neomys fodiens*. Scale bar = 10 μm .

Fig. 2. The trypanosomes *Trypanosoma ornata* sp. n. from water shrew *Neomys fodiens*. Scale bar = 10 μm .

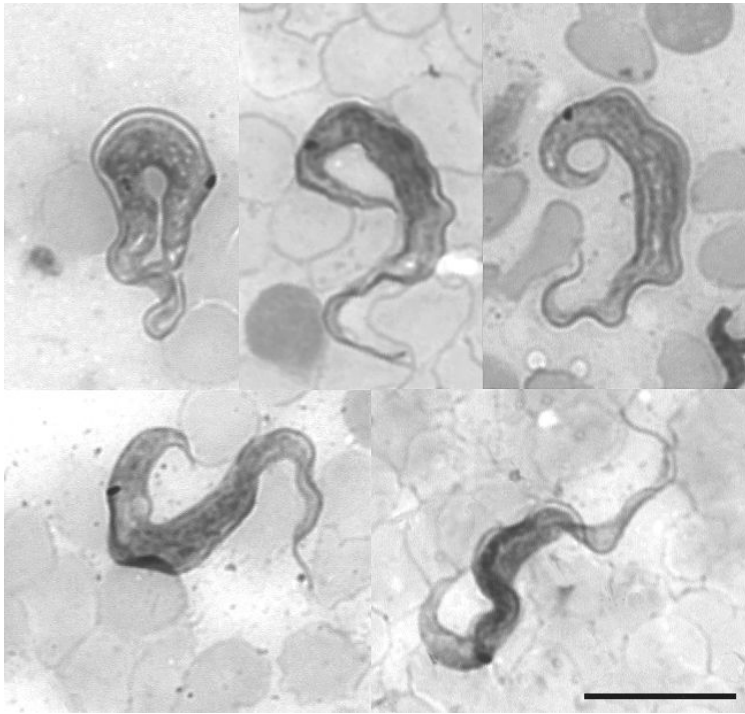


Fig. 1.

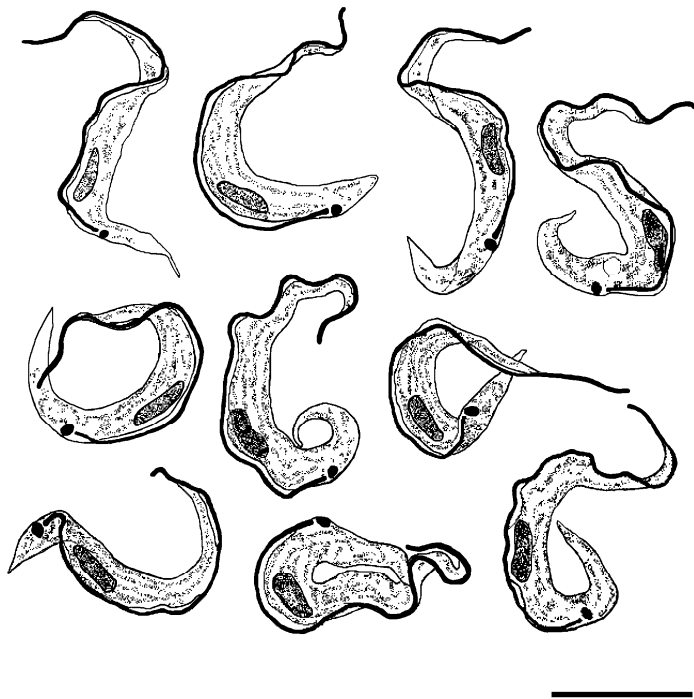


Fig. 2.